Discrepancies between two D-dimer assays and impact on clinical decisions; a retrospective analysis of samples tested in community- and hospital-based laboratories in Auckland

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ABSTRACT

AIM: In patients with suspected venous thromboembolism, an elevated D-dimer level provides an important branch-point in the management pathway. This study compared two D-dimer assays, INNOVANCE® DDimer (Innovance) and STA®-Liatest® D-Di Plus (Liatest), to assess potential impact on clinical management.

METHOD: Reflecting current practice in Waitematā, Auckland, we compared paired samples from 805 patients referred to hospital following a community D-dimer test. Samples were determined to be positive or negative using a 500µg/L fibrinogen equivalent units (FEU), and age-adjusted cut-offs.

RESULTS: In the Innovance assay, 2% of samples had a result <500µg/L FEU. In contrast, by Liatest, 18% were below 500µg/L. This positive bias of Innovance was amplified with use of age-adjusted cut-offs; 23% of samples with an elevated Innovance result showed a normal result by Liatest. On average, the Innovance values were 22% higher than Liatest. Results suggestive of interference from heterophile antibodies were seen in 6% of sample-pairs.

CONCLUSION: Innovance D-dimer test yielded higher values than Liatest and experienced interference from suspected heterophile antibodies. Discrepancies in nearly a quarter of patients may be leading to substantial under or over investigation, inefficient use of resources and clinical confusion.

In patients with suspected venous thromboembolism (VTE) a low D-dimer level is an important component of clinical algorithms that allows the safe discharge of patients with a low clinical probability score.[1] Although the decision-making cut-off points for D-dimer levels are internationally standardised, the assays themselves appear to show clinically important differences.

In the Auckland Region, patients in the community with suspected VTE undergo D-dimer testing at a community laboratory using the INNOVANCE® D Dimer assay (hereafter referred to as "Innovance"). If the D-dimer level is elevated, the patient may be referred to hospital for further investigation and re-tested by the hospital laboratory with the STA®-Liatest® D-Di Plus assay (hereafter "Liatest"). Laboratory staff at Auckland hospitals have observed that samples with high results from the Innovance assay can be normal when tested with the Liatest assay.[2,3] Laboratory staff have also received calls from general practitioners questioning the integrity of D-dimer testing when patients have discordant results.

D-dimers are degradation products of human blood coagulation, produced by dissolution of fibrin mesh. High levels of D-dimers are seen up to 20 days following a VTE, with laboratory D-dimer measurements considered most useful within 11 days of a suspected thrombotic event.[4]

The cut-off value used for classifying D-dimer levels as normal (negative) or high (positive) is often set at 500µg/L fibrinogen equivalent units (FEU).[1] As D-dimers positively correlate with age, cut-off values are frequently adjusted by raising the cutoff by 100µg/L FEU for each decade above 50 years.[5] The test has high sensitivity, meaning that either a level below 500µg/L or cut-off values

adjusted for age are useful for ruling out VTE; however, it lacks specificity.[1,6]

Many different quantitative D-dimer assays are available for laboratory testing and there is a lack of standardisation between them.[6,7] Large differences have been detected in patient samples and laboratory quality assurance surveys tested with different commercial kits.[8–10] In addition, there are numerous reports of falsely high positive D-dimer results caused by artefacts in patient samples, including lipaemia, some drug metabolites[11] and interfering antibodies including rheumatoid factor, anti-species and heterophile antibodies.[2,12–20] Heterophile is a blanket term for any antibody in the patient's plasma that can bind with low affinity to a range of naturally occurring antigens and antibodies, including the Fc portion of the monoclonal antibody used in an immunoassay.[21,22] Sources report heterophile antibody incidence in general populations in the range of 0.17% to 40%.[17] Despite the use of reagents to block these antibodies, interference can still be detected at reported rates of 0.05–0.5%.[17]

The aim of this investigation was to compare two commonly used assays in a real-world setting to assess systematic bias and heterophile antibody-like interference, and to assess the potential impact of discrepant D-dimers results on patient admissions and management.

Methods

This is a retrospective analysis of 818 paired samples collected from 805 individuals for D-dimer analysis over a 38-month period between January 2019 and March 2022, as part of routine healthcare. Each sample pair was collected within a 24-hour period, with a median time difference of 8 hours. The first of the sample pairs was collected in the community and analysed by the Innovance (Siemens) assay on a Sysmex CS-5100 platform. Patients with an age-adjusted elevated D-dimer result in the Innovance assay were referred to hospital, and a second sample was collected and tested by the Liatest (Stago) assay on a Stago STA R Max3. A small number of cases (2.6% of samples) with an Innovance result of <500µg/L FEU were retested in the hospital laboratory for other reasons. Females accounted for 68% of patients. Ages ranged from 14 to 102 years, with a median of 64 years.

D-dimer results were extracted for analysis from the laboratory management system. The upper limit of reporting in the Liatest assay in the hospital laboratory was 4,000µg/L FEU, whereas the community laboratory reported up to 40,000µg/L FEU with Innovance. Sample pairs with a result of >4,000µg/L FEU on Liatest (n=86) were removed from the dataset when analyses required numerical statistical comparison.

A cut-off of 500µg/L FEU for a positive or negative D-dimer result was applied to all samples for the first analysis of data. Subsequently, age-adjusted cut-offs were applied by raising the cutoff by 100µg/L FEU with each decade above 50 years of age. For example, for a patient in the age range 51–60 years, D-dimer was classified as negative when <600µg/L FEU.[5]

Statistical analysis was performed in Microsoft Excel. Sample pairs were analysed to compare numerical results with two different statistical tools: XY scattergram and Bland–Altman difference plots.

Results

In accord with the reasons for patients' hospital assessment, almost all (798; 98%) of the 818 paired samples had a D-dimer result at or above a single-point cut-off of 500µg/L FEU in the Innovance assay. In contrast, only 671 (82%) of the paired samples were above this cut-off with the Liatest assay.

Using age-specific cut-offs, 190 (23%) of the sample pairs with a positive Innovance result had a negative result value from the Liatest assay (Table 1). Reflecting the lower level of D-dimer in younger people,[5] a greater proportion of those under 50 years (34%) had a discordant result between Innovance and Liatest assays compared to the older age groups (Figure 1).

In addition to simply designating results as positive or negative, we compared quantifiable results from both assays. Of the 818 sample pairs, 86 with a value of ">4,000µg/L" from the Liatest were excluded, leaving 732 numerically comparable sample pairs. Of these 732, the median D-dimer result was 1,160µg/L FEU by Innovance, and 928µg/L FEU by Liatest.

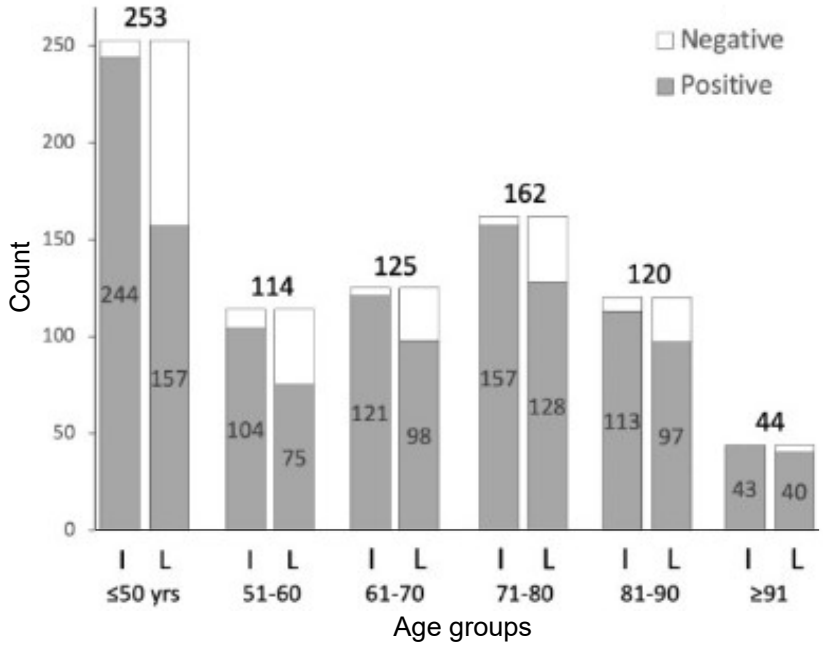
Results of these 732 pairs were converted to log base 10 to allow comparison, and are plotted in Figure 2.

Comparison of results revealed that 44 (6.0%) of 732 sample-pairs had values substantially higher (defined as 3-fold or greater) in Innovance than Liatest (Figure 2). It is suspected these 44 cases were affected by heterophile antibodies.[17] Due to the minimum reportable Liatest result of 270µg/L,

Table 1: Innovance versus Liatest using age-adjusted cut-offs (n=818).

	Innovance negative	Innovance positive	Totals
Liatest negative	33 (4%)	190 (23.2%)	223 (27.3%)
Liatest positive	3 (0.4%)	592 (72.4%)	595 (72.7%)
Totals	36 (4.4%)	782 (95.6%)	818

Figure 1: Comparison of Innovance (I) and Liatest (L) positivity by age group using age-adjusted cut-off values (n=818).



the Innovance result needed to be at least 810µg/L to exceed the 3-fold cut-off.

For further test comparison, we excluded the 44 highly discordant results where the Innovance result was greater than 3-fold higher than the Liatest result, leaving 688 paired results. Based on these 688 results, the relationship between the two assays was: Innovance = 1.25*Liatest + 22. On average the Innovance assay was 22% higher than the Liatest assay.

The Bland–Altman plot of these 688 cases shows a clear bias toward higher result in the Innovance assay (Figure 3). The mean positive bias was 336µg/L, but as shown there is a proportional bias, i.e., an increasing difference with increasing D-dimer values.

Given that the two sets of results were obtained on two different samples collected within 24 hours, it was possible that a biological change in D-dimer might have occurred between the two tests. No statistically significant difference was found between time elapsed and the two values (r^2=0.00002, p=0.91, Figure 4).

Discussion

This retrospective analysis of patient data provided a real-life opportunity to compare D-dimer results of 818 paired samples tested by two different immunoassays performed on two different platforms within a 24-hour time period. Among patients referred to hospital with a high

Figure 2: Comparison of D-dimer assay results between Innovance and Liatest. The tests were performed on separate samples within 24 hours for 732 sample pairs. Axes are displayed in logarithmic scale. The solid black line is the line of identity, and the dashed line shows where the Innovance result was 3-fold higher than the Liatest result.

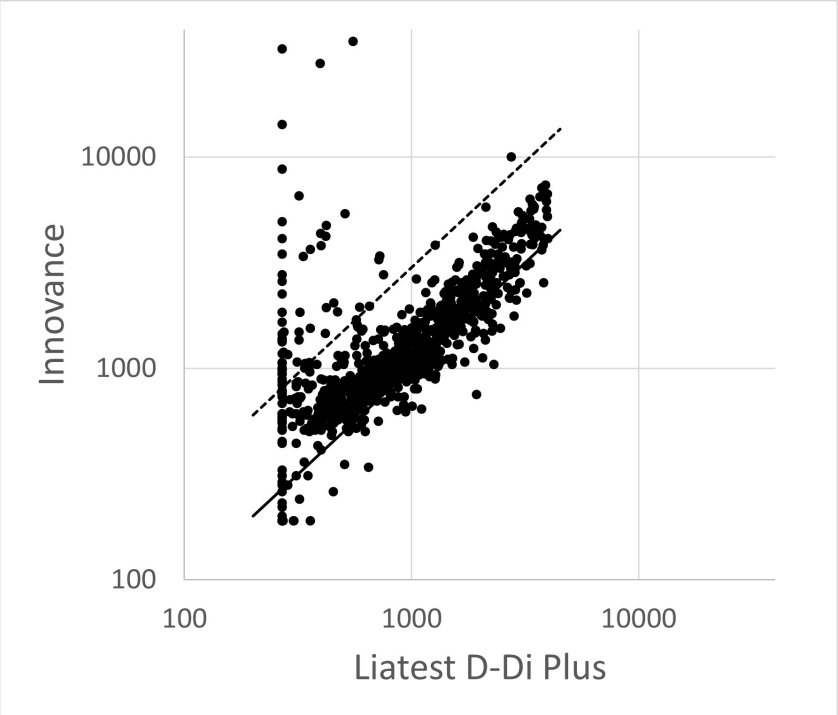


Figure 3: Bland–Altman plot of the difference between the Innovance and Liatest assays against the mean of the two assays. Lines represent the mean difference (central line), mean difference + 2SD (upper line) and mean difference—2SD (lower line).

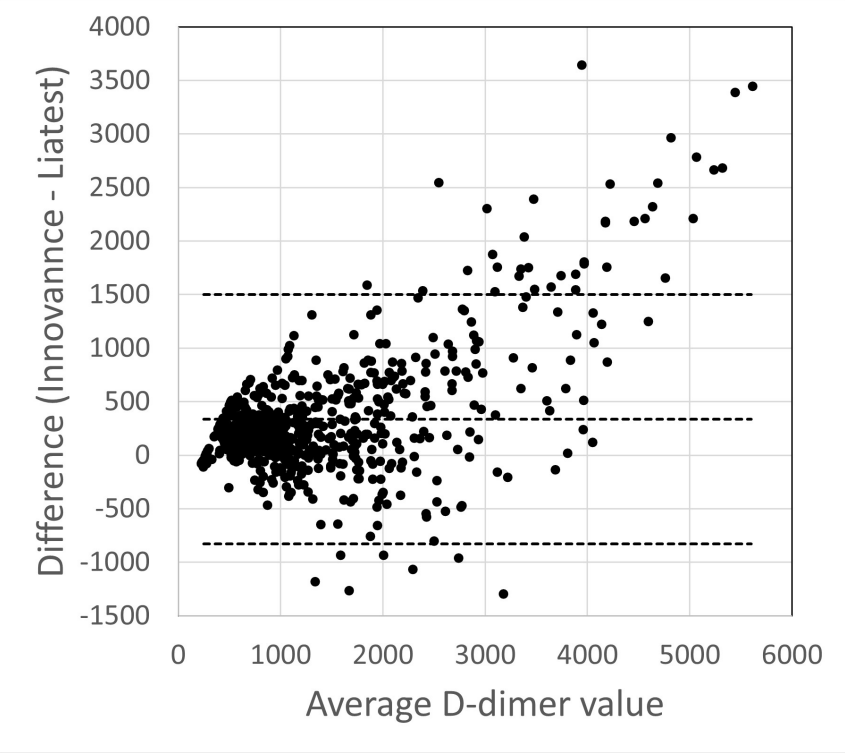
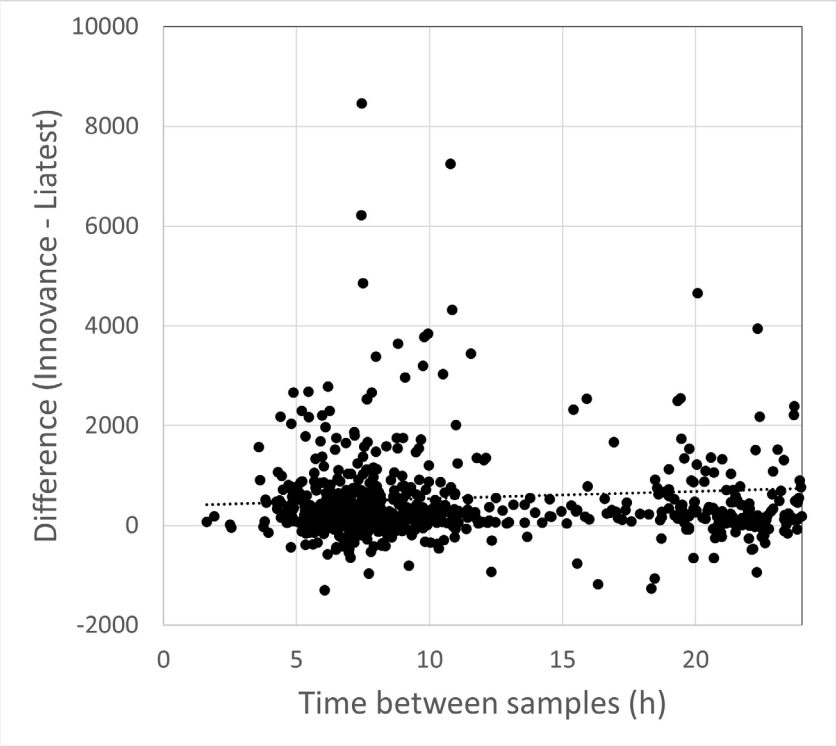


Figure 4: Comparison of difference between test results and elapsed time of the 732 comparable samples. Three extreme outliers with a difference of >13,000 were excluded from the analysis. The dashed line shows the line of best fit (r^2=0.00002, p=0.91, y=-0.59x + 448).



D-dimer result, 24% had a normal D-dimer when retested with the second assay. In the age group of 50 years and under (Figure 1), 35% of samples tested positive by Innovance but negative by Liatest. Six percent of total patients showed an Innovance D-dimer result at least 3-fold that of Liatest. The possibility that these differences were due to the difference in collection time, for reasons such as renal clearance or clot extension, was considered; however, there was no relationship between D-dimer level discrepancy and time elapsed between specimen collection (Figure 4). While we cannot completely exclude physiological differences in the two samples of each pair having an influence on results, others have observed the same positive bias of Innovance when testing the same sample by Innovance and Liatest.[8–10] Although comparison of the same samples/blood draws on the same methods is most scientifically accurate, this investigation reports on what is happening in a current real-world scenario.

Regardless of assay used, the same internationally standardised cut-offs are used to exclude thrombotic events, or to progress patients to further investigation.[1] The differences we have shown demonstrate that either cut-offs need to be individually established for each specific testing method, or assays must be standardised.[7]

Internationally, including Australasia, Innovance and Liatest are among the most commonly used D-dimer kits.[9,23] Discordance in D-dimer results between different assays (not restricted to Innovance versus Liatest) has been widely reported.[6,7,11] In an analysis of three quality survey samples, Favaloro and Thachil[10] found the median D-dimer result from participating laboratories that used the Innovance assay was approximately twice that of laboratories using Liatest. Hamer and colleagues[9] analysed D-dimer results from a survey sample sent to 645 different laboratories using a range of assays, and found that laboratories using the Innovance assay reported values 1.6 times higher than those using the Liatest assay. We observed a 22% difference in the mean D-dimer result in patients, with values from the Innovance assay consistently higher than from Liatest.

Antibodies present in patient samples leading

to false positive D-dimer results have previously been reported as isolated case reports,[2,14–20] but large-scale studies of the problem are limited[17] and may be under-reported. Here we report that 6% of D-dimer results may have been affected by heterophile and other interfering antibodies. This is in contrast to the rates of interference in immunoassays reported elsewhere as 0.05–0.5%.[17]

The problem of interfering antibodies occurs with various immunoassays, including D-dimer assays,[2,14–20] and causes diagnostic confusion, anxiety and sometimes unnecessary medical interventions for patients. Poor characterisation of the diversity of heterophile antibodies compounds the problem.[24]

Clinicians have a right to expect results for D-dimer tests to be consistent, regardless of assay manufacturer. As Hamer et al. commented, lack of D-dimer assay standardisation has "significant impact on costs, time and radiation exposure."[9] In nearly one quarter of patients in this study, measurement of D-dimer by a different assay may have led to altered clinical decision making. Discordant results erode trust in laboratory testing and healthcare providers. Currently, the Liatest assay is used to guide hospital-based management in Auckland hospitals, whereas the Innovance assay is widely used for community and hospital testing in other regions of New Zealand. It is unclear if either test provides more appropriate guidance for investigation and treatment in comparison to the international studies that were used to determine cut-off points for VTE clinical algorithms. Our study was retrospective and carried out in one region with a relatively small sample size. International cooperation must aim to standardise D-dimer assays to produce comparable results that are not prone to interference.

COMPETING INTERESTS
Nil.

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